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A Comparison of Ozone and Chlorine Toxicity to Three Life Stages of the American Oyster 'Crassostrea virginica'

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A COMPARISON OF OZONE AND CHLORINE TOXICITY TO THREE LIFE STAGES OF THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA*

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ABSTRACT

The effects of ozone-produced oxidants (OPO) on three developmental stages of the American oyster, Crassostrea virginica, *were evaluated. Both straight-hinge (48-h post fertilisation) and setting pediveliger larvae were exposed to a series of OPO concentrations ranging from 0·05 to 0·30 mg/litre OPO (as total residual chlorine) at 25°C for 96 h. Mortality was used as the response parameter and results were quantitatively compared with the effects of chlorine-produced oxidants (CPO) obtained from the literature on these same developmental stages. Adult oysters (2- to 4-year class) were exposed to OPO concentrations ranging from 0·01 to 0·50 mg/litre at 15°C for 5 days and to the same concentration range at 25°C for two consecutive 6-day periods. Shell deposition and faecal matter accumulation were used as the response parameters for this stage.*

Straight-hinge larvae were significantly more sensitive to OPO than setting pediveliger larvae. Both larval stages were significantly more sensitive to CPO than to OPO. Shell deposition was significantly inhibited at all OPO concentrations for both 15°C and 25°C acclimated adult oysters. No significant differences were found between the shell deposition response of oysters at the two acclimation temperatures. Faecal matter accumulation was significantly reduced at OPO concentrations ≥0·05 mg/litre at both 15°C and 25°C. It was significantly ($p < 0·05$) *greater for 25°C acclimated oysters than for 15°C acclimated oysters at OPO concentrations <0·10 mg/litre but not at higher concentrations.*

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INTRODUCTION

The use of chlorine for municipal and waste water disinfection and as an antifoulant in power plants is a common practice in the United States and other countries (Beauchamp, 1969; White, 1972; Jolley *et al.*, 1978). Chlorine and its residual by-products, however, are toxic to many forms of aquatic life (Brungs, 1976; Morgan & Carpenter, 1978; Hall *et al.*, 1981, 1982). Therefore, several alternative disinfectants and antifoulants have recently been considered (US Environmental Protection Agency, 1976; Burton & Linden, 1978; Garey, 1980). Ozone is one promising oxidant that has been proposed as an alternative to chlorine and is the focus of this study. The American oyster, *Crassostrea virginica*, was selected since it is a commercially important species and because its life cycle includes both sensitive planktonic larvae, which can become entrained in power plant cooling waters, and a sessile benthic adult, which would be unable to move away from the discharge area of treated effluents.

Recent investigations (Blogoslawski *et al.*, 1976; Williams *et al.*, 1978; Kosak-Channing & Helz, 1979; Helz, 1981; Richardson *et al.*, 1981) have shown that chlorination or ozonation of estuarine and marine waters differs from similar treatment of freshwater in that the Br^-, present in these saline waters, becomes oxidised to HOBr and OBr^-, although the exact speciation may be affected by complex organic material commonly found in natural waters. Thus, the toxicity of chlorinated or ozonated saline waters may be similar. To investigate this possibility the results of our ozone toxicity studies were compared with chlorine toxicity studies (Rhoderick *et al.*, 1977; Roosenburg *et al.*, 1980) performed with the same genetic oyster larvae stock and adult oysters using similar conditions and methodologies.

The terms ozone-produced oxidant (OPO) and chlorine-produced oxidant (CPO) are used throughout this paper to refer to the amperometrically detectable total resident oxidant concentrations (measured as total residual chlorine) produced in solution following ozonation or chlorination, respectively. Thus, the term CPO is synonymous with the more commonly used terms, total residual chlorine (TRC) and total residual oxidant (TRO).

MATERIALS AND METHODS

Larval experiments

Spawning and rearing: Adult *Crassostrea virginica*, 2–6 years old, were obtained from the Wilde Oyster Hatchery, West River, Maryland. Oysters were scrubbed clean and placed in 1·2 × 2·4 × 0·2 m holding trays supplied with a continuous flow of 12 °C filtered Patuxent River water (≃12‰). Over a six-week period the temperature was gradually increased from 12 °C to 20 °C using 1000 W Vycor heating elements. Oysters were fed daily with *Isochrysis galbana*. Spawning was

induced by a sudden increase in both temperature and food supply as described by Loosanoff & Davis (1963). Spawning oysters were removed from the spawning tray, sorted by sex and the sperm and eggs collected in separate tanks. Fertilisation was initiated by mixing sperm and eggs and allowing them to remain in contact for 10–15 min. The end of this contact period was considered to be the initiation of development (i.e. time 0).

After screening and washing, the density of the fertilised eggs was adjusted to 100 per millilitre by adding 1-μ filtered Patuxent River water ($\simeq$12‰). During the first 48 h following fertilisation, the developing larvae were not fed. At 48 h post-fertilisation, larvae had reached the early straight-hinge stage of development. Larval concentration was again adjusted to 100 per millilitre and aliquots were pipetted into experimental chambers for testing. The remaining larvae in the stock culture were screened and washed through a 45 μ mesh screen, transferred to 19 litres of 25 °C, 5-μ filtered estuarine ($\simeq$12‰) water, fed with *Isochrysis galbana* and then treated with 200 mg/litre of Sulmet antibiotic. Screening, water changing, feeding and adding antibiotics were performed on a daily basis for the stock culture. Larval density decreased from $\simeq$100 per millilitre to $\simeq$10 per millilitre during the 9 to 21 days required for larvae to reach the pediveliger stage of development. When most of the pediveliger larvae had developed an eye spot and had begun setting they were concentrated to 100 per millilitre and transferred to a shallow setting tray. This tray was lined with black polyethylene sheeting and the entire bottom covered with numbered 2·5 × 7·6-cm glass microscope slides, which were used as experimental substrates. When the density of setting pediveliger larvae reached 100–200 per slide, each slide was removed and counted for the total number of live and dead larvae before being placed into an experimental chamber. Pediveliger larvae were in the late crawling stage. They had not permanently cemented to the slide nor undergone metamorphosis.

Experimental procedure: Ozone gas was produced with an electrical discharge type ozonator (Model T-816, Welsbach Ozone Systems Corp., Philadelphia, PA) using dry aviator oxygen. The ozone–oxygen mixture ($\simeq$4% O_3 by volume) was bubbled into the base of an 11·5-litre, polycarbonate countercurrent contact column (7 cm inside diameter; 2·75 m high) at 1·5 litre /min through a 3-cm fritted glass disc. Filtered (5 μ) Patuxent River water was pumped into the top of the column whilst ozonated water was drawn from the bottom and fed to four glass rotameters for metering into the experimental apparatus.

Filtered water from a constant pressure head tank was continuously delivered to five 1-litre distribution tanks, through 0·6 cm inside diameter glass tubing. Four of the five delivery tubes were fitted with 'tees', through which ozonated water from the rotameters could be injected. No oxidant was injected into the fifth delivery tube, which supplied the control distribution tank. Each 1-litre distribution tank was equipped with ten supply lines, each of which supplied a continuous flow ($\simeq$20 ml/min) of test solution to a separate experimental chamber. Experimental

chambers were constructed from 20 cm lengths of 7 cm inside diameter glass tube with 35-μ nitex mesh attached to one end with silicone aquarium sealer. All ten chambers at each concentration were placed in a 10-cm deep fibreglass tray to maintain an adequate water level ($\simeq$5 cm) in the chambers.

Ozone-produced oxidant (OPO) concentrations were measured amperometrically as equivalent total residual chlorine (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1976) using a PAR polarographic analyser (Model 174A; Princeton Applied Research Corp., Princeton, NJ) equipped with a 10-g rotating spiral platinum electrode and a standard platinum disc reference electrode. Phenylarsine oxide ($2{\cdot}82 \times 10^{-3}$ N) was used as the titrant and was delivered with a syringe microburette (Model SB2; Micro-Metric Instrument Co., Cleveland, OH) capable of titrant additions as small as 3 μl. The potassium iodide and pH 4 reagents were prepared according to *Standard Methods* (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1976) and were premixed in the bottom of a 200 ml volumetric sample collection flask to minimise possible underestimation of the oxidant concentration (Carpenter *et al.*, 1977; Crecelius *et al.*, 1978).

At the beginning of the straight-hinge larval experiment, 2 ml of the 100 larvae per millilitre stock culture were pipetted into each of the experimental and control chambers yielding a total $\simeq$200 larvae per chamber ($\simeq$one larvae per millilitre of test solution). Ten chambers were simultaneously started at each concentration of 0·000, 0·036 ($\pm$0·013), 0·098 ($\pm$0·024), 0·184 ($\pm$0·047) and 0·318 ($\pm$0·033) mg/litre OPO. These concentrations were selected following the results of preliminary range finding experiments conducted with larvae from a previous spawn. Duplicates (one pair) were removed from each concentration after 12, 24, 48 and 96 h of exposure. Upon removal, chambers were placed in a bath of 0·1 % neutral red dye for 15 min, then rinsed and washed through a 10-μ mesh collecting cup and placed in a Sedgewick-Rafter cell where mortality counts were made for each chamber under 300 $\times$ magnification.

The neutral red dye acted as a 'vital stain' and was picked up by the tissues of living larvae, causing them to appear pink. This aided determination of live or dead larvae. Additional death criteria were empty shells, caused by bacterial action, and lack of movement. Water quality during the straight-hinge larvae experiments was: temperature, 25 °C; salinity, 11·9‰; pH, 7·8; dissolved oxygen concentrations of 7·4, 9·0, 10·4, 11·2 and 11·6 mg/litre for nominal OPO concentrations of 0·00, 0·05, 0·10, 0·20 and 0·30 mg/litre, respectively.

In the setting pediveliger larval experiments, glass microscope slides with approximately 200 larvae attached to one side were obtained from the setting tray described earlier. Most of the setting pediveligers were in the late crawling stage and very few were permanently cemented (Galtsoff, 1964; Cranfield, 1973). No larvae had reached the metamorphosis stage of development at the beginning of the exposure period although some did undergo metamorphosis by the end of the

exposure period. After counting to determine the original number of live and dead larvae, each slide was placed into an experimental chamber identical to those used in the straight-hinge study. Ten chambers were placed in each OPO concentration of 0·000, 0·061 (±0·024), 0·105 (±0·048), 0·180 (±0·055) and 0·277 (±0·064) mg/litre; duplicates were removed from each concentration after 12, 24, 48 and 96 h of exposure to determine mortality. Concentrations and exposure times were selected to cover the same ranges used in the straight-hinge experiments. At each exposure time all slides which had been removed were placed in a staining bath of 0·1% neutral red dye for 15 min, then examined under 300× magnification to determine the total number of live and dead larvae. Criteria for death were the same as for the straight-hinge larvae with the exception of movement. Water quality during this study was: temperature, 25°C; salinity, 11·9‰; pH, 7·4; dissolved oxygen concentrations of 7·8, 8·8, 9·6, 10·7 and 11·1 mg/litre for nominal OPO concentrations of 0·00, 0·05, 0·10, 0·20 and 0·30 mg/litre, respectively.

Statistical analyses: Response surface models for both straight-hinge and setting pediveliger larvae were derived where percent mortality was represented by a function of oxidant concentration and exposure time. Three transformations (log, logit and arcsine) of the original mortality data were tested by the stepwise procedure of SAS (SAS Institute Inc., 1979). A model using the arcsine transformation was selected since it consistently yielded the simplest model with the highest degree of accuracy. The following linear model was initially introduced to the stepwise procedure for this transformation.

$$\begin{aligned}\text{Arcsine}\,[(r/n)^{0\cdot5}] = {} & A + B\,\text{conc} + C\,\text{time} + D\,\text{dose} + E\,\text{conc}^2 \\ & + F\,\text{time}^2 + G\,\text{dose conc} + H\,\text{dose time} \\ & + I\,\text{dose}^2 + J\log(\text{conc}) + K\log(\text{time})\end{aligned}$$

where: conc = exposure concentration (mg/litre), time = exposure time (h), dose = conc × time, r = mortality response for a given dose, n = sample size at a given dose and A–K = coefficients estimated by least squares procedure.

Final models were selected based on the significance level ($p < 0{\cdot}05$) of a variable's contribution and the simplicity of the equation. A comparison was made between these response surfaces to determine if there were a difference in the resistance of the two developmental stages to OPO. The difference between the relative resistance of straight-hinge and setting pediveliger larvae to OPO and CPO (chlorine-produced oxidants) was also tested by comparing our response surface models with similar ones obtained from the literature (Roosenberg *et al.*, 1980). All response surface comparisons were performed with standard analysis of variance procedures (Sokal & Rohlf, 1969).

Adult experiments

Cultchless, 1- to 4-year old oysters (*Crassostrea virginica*) were obtained from the Wilde Oyster Hatchery, West River, Maryland, and acclimated to either 15°C or

25 °C in large holding trays (1·5 × 3·0 × 0·2 m) supplied with continuously flowing Patuxent River water (≃9‰) for 3 weeks prior to experimentation. These acclimation temperatures were selected as representative of temperatures near the high and low limits encountered during the seasonal periods in which anti-fouling chlorination is conducted. In addition, temperatures below 15 °C would have required an extended exposure period in order to obtain a measurable change in faecal accumulation and shell deposition. During acclimation oysters were scrubbed clean at least once a week and fed daily with *Isochrysis galbana*. Production of various OPO concentrations was similar to the method used in the larval experiments. However, the Patuxent River water was not filtered, flow rates were much greater (200 ml/min) and 7-litre fibreglass hatchery trays (Heath Tecna Corp., Kent, WA) were used as experimental chambers.

Experimental procedure: Groups of twelve oysters (mean length = 7·5 ± 0·8 cm), acclimated to 25 °C, were exposed to each OPO concentration of 0·000, 0·009 (±0·000), 0·046 (±0·002), 0·094 (±0·004), 0·174 (±0·006), 0·301 (±0·027) and 0·483 (±0·022) mg/litre for two consecutive 6-day periods. Shell deposition and faecal accumulation were measured twice following 6 and 12 days of exposure. A paired *t*-test showed no significant ($p < 0·05$) differences in either the shell deposition or faecal accumulation responses between the first and second 6-day periods of exposure.

Shell deposition was measured according to *Standard Methods* (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1976). Faecal accumulation was measured as a total accumulation for all oysters at each concentration and was quantified in the following manner. At the termination of an experiment, all accumulated faecal matter (faeces and pseudofaeces) was transferred to a large plastic container after removing the oysters and allowed to settle for 48 h. The supernatant was then siphoned off, the precipitate concentrated in a glass container and the volume adjusted to exactly 4 litres. After homogenisation a 100-ml aliquot was removed and filtered through preweighed No. 2 Wattman filter paper. It was then dried at 100 °C for 24 h and reweighed to obtain a faecal accumulation coefficient for each OPO concentration.

Groups of ten oysters, acclimated to 15 °C, were exposed to each OPO concentration of 0·000, 0·012 (±0·002), 0·052 (±0·016), 0·105 (±0·037), 0·205 (±0·047), 0·293 (±0·077) and 0·490 (±0·166) mg/litre for 120 h. Because there was no difference in the response parameters at 25 °C between the first and second 6-day periods of exposure, the study at 15 °C was planned for only one 6-day period. Unfortunately, a power failure occurred during the night between the 120- and 132-h check times. As adequate time and oysters were not available to repeat the experiment, the shell deposition and faecal accumulation were measured following the 120-h exposure. Oysters tested had a mean length of 6·7 (±0·7) cm. Water quality was: temperature, 16 °C; salinity, 9·2‰; pH, 8·0; turbidity, 1·57 NTU;

dissolved oxygen concentrations of 9·6, 10·3, 11·3, 11·9, 12·8, 13·6 and 14·6 mg/litre, for nominal OPO concentrations of 0·00, 0·01, 0·05, 0·10, 0·20, 0·30 and 0·50 mg/litre, respectively.

Statistical analyses: Shell deposition was analysed at each acclimation temperature with an analysis of variance and Duncan's multiple range test, while faecal accumulation was analysed using regression techniques. The mean shell deposition and faecal accumulation values for the first and second 6-day periods were utilised for the 25 °C experiment since there were no significant differences. For comparisons between the two acclimation temperatures (15 °C and 25 °C) it was necessary to normalise the data since the results at 25 °C were for a 6-day test whilst the results at 15 °C were for a 5-day test. Therefore, the response parameters at 15 °C and 25 °C were divided by their respective exposure periods (5 and 6 days) and then multiplied by 4 to obtain a normalised response based on a standard 96-h exposure. Differences between these normalised response parameters at the two acclimation temperatures were tested with a standard *t*-test.

RESULTS

Larval experiments

The final predictive equations for the toxicity of OPO to straight-hinge and setting pediveliger larval stages are shown in Table 1. Response surfaces (Figs 1 and 2) were drawn from these equations after transforming the data according to the equation:

$$\text{Predicted per cent mortality} = (\sin Y)^2 \times 100$$

The predictive equations from Roosenburg *et al.* (1980) are also shown in Table 1. The single most important factor in predicting the mortality of both larval stages exposed to each oxidant was found to be the dose (i.e. concentration × time). This indicates that the mode of action is similar for both OPO and CPO. Setting pediveliger larvae, however, are significantly more resistant to these oxidants than

TABLE 1

PREDICTIVE RESPONSE MODELS FOR THE MORTALITY OF STRAIGHT-HINGE AND PEDIVELIGER LARVAE OF THE OYSTER (*Crassostrea virginica*) EXPOSED TO A SERIES OF OPO AND CPO CONCENTRATIONS FOR PERIODS UP TO 96 h

Developmental stage	*Oxidant*	*Modelling equation*
Straight-hinge larvae	OPO	$Y = 0{\cdot}1247 + 0{\cdot}0186D + 0{\cdot}004DT$
Straight-hinge larvae[a]	CPO	$Y = 0{\cdot}2385 + 0{\cdot}1090D - 0{\cdot}2370DC$
Pediveliger larvae	OPO	$Y = -0{\cdot}0612 + 0{\cdot}0086D + 0{\cdot}0831(\ln T)$
Pediveliger larvae[a]	CPO	$Y = 0{\cdot}1989 + 0{\cdot}6609C + 0{\cdot}0344D - 0{\cdot}0009D^2$

[a] From Roosenburg *et al.* (1980).

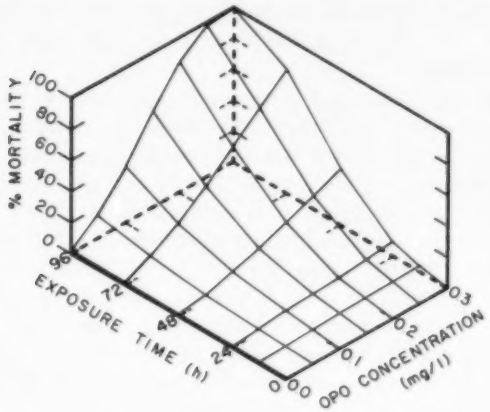

Fig. 1. Response surface for the mortality of straight-hinge larvae exposed to a series of OPO concentrations for periods up to 96 h.

straight-hinge larvae (Table 2; Comparisons I and II). CPO was found to be significantly more toxic than OPO to both developmental stages (Table 2; Comparisons III and IV) even though the response surfaces appeared to be quite similar. This relationship can also be seen by comparing LC_{50} values from our work with those of Roosenburg *et al.* (1980). They reported 48-, 72- and 96-h LC_{50}'s of 0·30, 0·08 and 0·06 mg/litre CPO, respectively for straight-hinge larvae, whilst the 48-, 72- and 96-h LC_{50}'s in our studies were 0·36, 0·19 and 0·12 mg/litre OPO,

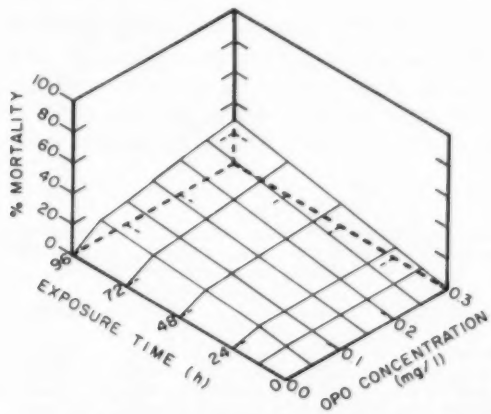

Fig. 2. Response surface for the mortality of setting pediveliger larvae exposed to a series of OPO concentrations for periods up to 96 h.

TABLE 2
ANOVA STATISTICS FOR COMPARISON OF RESPONSE SURFACE MODELS ACROSS BOTH LIFE STAGES (STRAIGHT-HINGE, PEDIVELIGER) OF THE OYSTER (*Crassostrea virginica*) AND OXIDANTS (OPO, CPO).

Comparison	*Source*	*df*[a]	MS[b]	F[c]	*PR* > F[d]
I OPO: straight-hinge–pediveliger	Life stage	8	0·203	13·682	0·001
	Error	183	0·015		
II CPO: straight-hinge–pediveliger[e]	Life stage	8	0·297	20·013	0·001
	Error	183	0·015		
III Straight hinge: OPO–CPO[e]	Oxidant	8	0·087	5·840	0·001
	Error	183	0·015		
IV Pediveliger: OPO–CPO[e]	Oxidant	8	0·188	12·689	0·001
	Error	183	0·015		

[a] Degrees of freedom
[b] Mean square.
[c] *F*-statistic.
[d] Significance level.
[e] From Roosenburg *et al.* (1980).

respectively. LC_{50} values were not computed for setting pediveliger larvae since the maximum mortality never reached 50% and extrapolation of an arcsine or probit model would predict an LC_{50} much higher than the true value. The highest mortality for pediveliger larvae exposed to the maximum doses of OPO and CPO was 29·0% and 43·5%, respectively.

Adult experiments

The results of the shell deposition experiment are shown in Table 3. Shell deposition values (normalised) for 15 °C and 25 °C were not significantly different at the 0·05 level for all exposure concentrations. The Duncan's multiple range analysis is shown in Table 3 by the line underscoring all concentrations at which shell

TABLE 3
SHELL DEPOSITION[a] RESULTS FOR ADULT OYSTERS (*Crassostrea virginica*) EXPOSED TO A SERIES OF OPO CONCENTRATIONS AT 15 °C AND 25 °C (SEE TEXT FOR DETAILS).

Temperature	*Parameter*	*Nominal OPO concentration (mg/litre)*						
		0·00	*0·01*	*0·05*	*0·10*	*0·20*	*0·30*	*0·50*
15 °C	Mean (mm)	0·93	0·38	0·26	0·20	0·08	0·11	0·04
	± SD	0·22	0·14	0·06	0·07	0·04	0·04	0·03
	% of control	—	41	28	22	9	12	4
25 °C	Mean (mm)	0·96	0·22	0·30	0·06	0·11	0·04	0·06
	± SD	0·26	0·09	0·11	0·02	0·07	0·02	0·06
	% of control	—	23	31	6	11	4	6

[a] Normalised for a 96-h exposure.

depositions were not significantly different ($p < 0.05$). As can be seen from the Table, the same response pattern is seen at both 15 °C and 25 °C, where the shell deposition for control oysters is significantly greater than all exposure concentrations and where there is no significant difference between any of the OPO exposure concentrations.

Faecal accumulation for oysters exposed to the same OPO concentrations at 15 °C and 25 °C is shown in Table 4. Faecal accumulation at both temperatures was

TABLE 4

FAECAL ACCUMULATION RESULTS FOR ADULT OYSTERS (*Crassostrea virginica*) EXPOSED TO A SERIES OF OPO CONCENTRATIONS AT 15 °C AND 25 °C (SEE TEXT FOR DETAILS).

Temperature	*Parameter*	*Nominal OPO Concentrations (mg/litre)*						
		0·00	*0·01*	*0·05*	*0·10*	*0·20*	*0·30*	*0·50*
15 °C	mg/g wet weight[a]	26·0	27·6	19·2	13·6	11·2	12·0	10·8
	% of control	—	106	73	53	42	45	41
25 °C	mg/g wet weight[a]	77·6	68·2	31·8	20·6	10·9	12·2	9·2
	% of control	—	88	41	26	14	16	12

[a] Expressed as: milligrammes of faecal matter per gramme of wet oyster weight per 96 h.

greatest in the control group and decreased as the concentration increased for OPO-exposed groups. The relationship between reduced faecal matter accumulation and OPO concentration, following regression analysis, was found to be:

$$15\,^{\circ}\mathrm{C} \quad F = 8{\cdot}078 + 1{\cdot}116 \div (\mathrm{conc} + 0{\cdot}0562)$$

$$25\,^{\circ}\mathrm{C} \quad F = 10{\cdot}562 + 1{\cdot}078 \div (\mathrm{conc} + 0{\cdot}0143)$$

where: F = faecal accumulation (mg/g total wet oyster weight/96 h) and conc = OPO exposure concentration (mg/litre).

A paired t-test showed no significant ($p < 0.05$) difference between the normalised faecal accumulation at 15 °C and 25 °C for OPO concentrations greater than $\simeq 0{\cdot}10$ mg/litre. However, faecal accumulation for 15 °C acclimated oysters was significantly lower than for 25 °C acclimated oysters at concentrations $< 0{\cdot}10$ mg/litre OPO. Approximate EC_{50} values for the 15 °C and 25 °C faecal accumulation experiments were 0·13 and 0·02 mg/litre OPO, respectively.

DISCUSSION

The results described above are the only quantitative data we have seen in the literature for the direct toxicity of OPO to the larval stages of *C. virginica*. DeManche *et al.* (1975) qualitatively described the effectiveness of using various techniques to remove the residual toxicity of ozonised sea water to oyster larvae but

did not measure the residual oxidant levels. MacLean *et al.* (1973) studied the effects of ozone-treated sea water on the spawned, fertilised, meiotic and cleaving eggs of *C. virginica*. Although they did not measure residual oxidant concentrations during the experiment, they estimated that the residual concentration may have been ≃0·18 mg/litre OPO at the start of the experiment. It is, therefore, not surprising that they found a severe impact on these extremely sensitive developmental stages. Stewart *et al.* (1979) conducted 48-h static bioassays on fertilised eggs of *C. virginica* using three by-products which can be formed following ozonation or chlorination. They found a marked decrease in survival using chloroform, bromoform and bromate in concentrations as low as 0·05–0·10 mg/litre. Unfortunately, we did not measure these during the course of this study. However, later experimentation indicated that bromate formation does not occur to any great extent in natural waters due to the presence of aminated and complex organic compounds (Richardson *et al.*, 1981).

More work has been conducted on the toxicity of CPO to larval oysters. Although the responses of oyster larvae exposed to OPO and CPO were statistically different (Table 2), the response surface configurations appeared to be quite similar for the two oxidants. It is possible, when fitting a polynomial response surface model over a sparse set of independent variables, for the predicted model to reflect anomalies in the data that could have resulted only from random deviation from the mechanism that controls mortality. When this occurs the model 'overfits' the data. Therefore, a high goodness of fit for each life stage–oxidant model and the relatively small number of independent variables in each experiment may have produced a significant difference due to 'overfitting' of the response surfaces. It is also possible that the slightly lower mortality of larvae exposed to OPO is due to the elevated dissolved oxygen concentrations. The dissolved oxygen concentrations in our test solutions were directly related to the OPO concentration. Higher concentrations contained greater amounts of ozonated water which became supersaturated with oxygen in the contact column. This condition resulted in dissolved oxygen levels of as much as 150% saturation at the highest OPO test concentrations. The major mechanism of toxicity of both ozone and chlorine to fish is through the destruction of gill epithelium (Wedemeyer *et al.*, 1979; Middaugh *et al.*, 1980). If a similar mechanism is the cause of mortality in oyster larvae, elevated dissolved oxygen concentrations would increase the efficiency of the oxygen transfer at the gill surface. Thus, larvae could survive a greater amount of tissue destruction in ozonated water before death through asphyxiation. This hypothesis is partially supported by work at this laboratory showing a significant difference in survival of adult white perch (*Morone americana*) exposed to the same OPO concentration at different dissolved oxygen concentrations (unpublished data). Further studies are needed, however, to support this hypothesis for oyster larvae.

Our results also agree with the data from Waugh (1964) who found that short exposures (3–20 min) to high CPO concentrations had a negligible effect on early

Ostrea edulis larvae at concentrations up to 10 mg/litre. Roberts & Gleeson (1978), however, showed that the 48-h LC_{50} for *C. virginica* larvae during the trocophore stage was ≃0·02–0·03 mg/litre CPO. The trocophore stage, therefore, is an order of magnitude more sensitive to both OPO and CPO than the early straight-hinge stage, while pediveliger larvae are even less sensitive than the straight-hinge larvae. These data clearly show that the sensitivity of larval oysters to CPO and OPO is greatest immediately following fertilisation and decreases with larval age up to the pediveliger stage of larval development.

Once larvae have set and metamorphosed, the adult form also becomes progressively more tolerant with age to the presence of residual oxidants. Our studies and others (Rhoderick *et al.*, 1977; Liden *et al.*, 1980) have shown that adult oysters, at least 1 year old, are capable of surviving 1- to 2-week exposures to high OPO and CPO concentrations. Therefore, more sensitive physiological responses were investigated in this study. Both shell deposition and faecal matter accumulation have been shown to be sensitive indicators of an oyster's condition (Scott & Middaugh, 1978; Liden *et al.*, 1980; Sheridan, in press). Our work shows that at residual oxidant concentrations greater than ≃0·20 mg/litre OPO, oysters remain closed almost constantly and neither feed nor produce faecal matter. Death would thus result from the indirect causes of starvation or asphyxiation if the exposure time exceeded an oyster's ability to sustain itself through anaerobic metabolism. Below 0·10 mg/litre OPO oysters began to produce more faecal matter as concentrations became smaller and remained open for longer periods (qualitative observation). These faecal accumulation data agree with those of Scott & Middaugh (1978) who found significant reduction in faecal production in oysters exposed to 0·12 mg/litre CPO or higher concentrations. Shell deposition, however, was affected at even lower concentrations (0·01 mg/litre OPO). Although the exact EC_{50} was not calculated, our data indicate that it lies below 0·01 mg/litre OPO. This estimate is supported by Sheridan (in press) who found growth of *C. virginica* spat to be unaffected at ≃0·003 mg/litre CPO and significantly reduced at ≃0·01 mg/litre CPO.

Thus, the growth of oysters continuously exposed to OPO or CPO concentrations of 0·01 mg/litre or more for extended periods may be strongly inhibited. Concentrations ≥0·05 mg/litre would result in almost no growth, poor oyster quality and, therefore, the eventual decline of the exposed population. These assumptions, however, are based on a continuous exposure with water quality similar to that in this study; intermittent or periodic exposure would probably result in a markedly lower impact. Variations in water quality can also drastically modify the specification and reaction paths of residual oxidants and their by-products in marine or estuarine water. Preliminary investigations at this laboratory have shown that identical amperometrically measured residual oxidant concentrations can produce significantly different toxicities in water from different locations. Extensive work is needed in the area of marine and estuarine water chemistry following

ozonation or chlorination, to identify which end-products are most toxic and how their formation is influenced by the complex array of physical and chemical parameters in natural waters.

CONCLUSIONS

(1) The effects of OPO on all life stages of the American oyster, *Crassostrea virginica*, are similar to the effects of CPO.
(2) *Crassostrea virginica* is most sensitive to OPO during the early larval stages and becomes increasingly more resistant as development progresses.
(3) Adult oysters are extremely resistant to OPO when mortality is the response, but both shell deposition and faecal matter accumulation are significantly reduced at concentrations as low as 0·01 and 0·05 mg/litre OPO, respectively.
(4) Temperatures between 15 °C and 25 °C do not affect shell deposition and faecal accumulation responses of adult oysters to OPO.
(5) Oyster larvae from early straight-hinge to the pediveliger stage could survive short-term (i.e. < 24 h) exposure to concentrations $\leq 0{\cdot}3$ mg/litre OPO without significant mortality.
(6) Adult oysters located in the receiving waters of a continuously ozonated or chlorinated discharge, where residual levels of 0·01 to 0·05 mg/litre OPO are present, may exhibit growth inhibition and a general lowering of physiological condition, depending on the physical and chemical characteristics of the ambient water.

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